Underlying Mechanisms of Alcohol-Induced Damage to the Fetus

Research has firmly established that maternal alcohol consumption can lead to fetal alcohol syndrome (FAS), a disorder defined in the early 1970's that is characterized by brain damage, physical defects of the face (called craniofacial defects), and growth deficiency. Current research on FAS seeks to delineate the specific mechanisms of damage to the fetus as well as the conditions that influence the extent of this damage.

Numerous factors complicate this research. First, the process of development itself is enormously complex and not yet fully understood (see the box, below, and figure 1). Second, because no single mechanism can account for the variety of structural, functional, and behavioral problems found in FAS, scientists believe that a number of distinct mechanisms work simultaneously along different biochemical pathways and at different

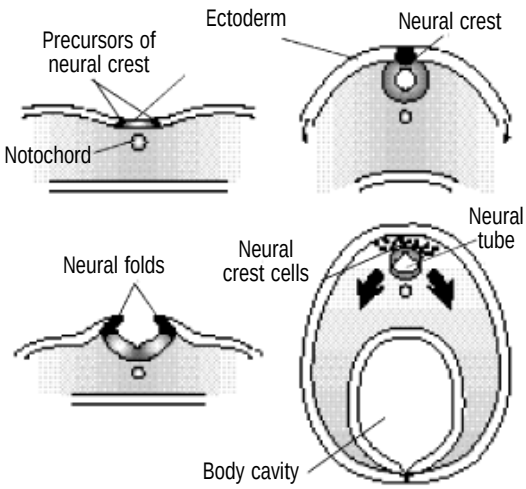
Embryonic Development of the Nervous System

Embryonic development is basically the same in all vertebrates, although the timing of developmental stages varies among species. In the human embryo, about 2 weeks after fertilization, in a process called gastrulation, the embryo develops three distinct layers: the ectoderm, the endoderm, and the mesoderm. These undifferentiated cell layers are eventually transformed into different body structures. The ectoderm produces the skin and nervous system; the endoderm forms the lining of the digestive tract, respiratory tubes, and associated organs; and the mesoderm produces the cardiovascular system, bones, muscles, and connective tissue.

In the early development of the nervous system, at about 3 weeks after fertilization in the human embryo, a strip of cells in the mesoderm (the notochord, which will eventually form the spinal column) induces the ectoderm above it to fold upward, where it forms two ridges at the embryo's midline. The tops of these ridges, known as the neural fold, then curve inward, and by the fourth week the ridge tops meet and fuse to form a tube. This is the neural tube, which will eventually form the brain and spinal cord. Cells originating from the fused tips of the neural fold are called neural crest cells. These cells migrate to specific locations in the embryo, where they differentiate into specific types of cells and begin to form a variety of body structures. By the end of the eighth week, the elements of all major body systems are in place.

Cells of the neural crest are particularly sensitive to alcohol-induced injury and death. Therefore, FAS research has focused on these cells, particularly on a subset of cells known as cranial neural crest cells, which give rise to facial cartilage and bone as well as many other body structures. The eventual fate of the cranial neural crest cells—the specific body structure and type of tissue that they will develop into—is predetermined before the cells begin migration. Another subset of cells, the trunk neural crest cells, gives rise to elements of the peripheral nervous system. These cells differ from the cranial neural crest cells in that their eventual fate is not predetermined before migration, but depends upon the environment into which they migrate. The trunk neural crest cells seem to be less affected by alcohol than the cranial neural crest cells, which may reflect their greater adaptability.

Figure 1: Origin of the nervous system



Early in embryonic development, the ectoderm directly above the notochord begins to form the primitive nervous system (the neurepithelium) (A). The neurepithelium then curves upward to form two ridges, called the neural folds (B). The tips of the neural folds fuse to form the neural tube, and cells from the fused tips of the neural folds form the neural crest (C). The neural crest cells migrate to various locations within the embryo, where they will initiate the development of various body structures (D).

Source: Smith 1997.

physical sites in the developing embryo. And third, the ways in which these alcohol-induced mechanisms produce damage to the fetus depend on several variables, including the timing, frequency, and amount of maternal drinking during pregnancy; the mother's health status and habits; and the genetic makeup of the mother and fetus.

Despite the complexity of FAS, scientists have made significant progress in defining its underlying mechanisms in recent years. This section first describes the multifaceted aspects of FAS that challenge research in this area, then presents findings from current studies, focusing on mechanisms of damage to the brain and craniofacial region.

Challenges to FAS Research: Multiple Mechanisms, Sites of Action, and Risk Factors

Research on FAS has shown that alcohol exerts its effects on the developing fetus through multiple actions at different sites (Abel 1990, 1995; Diamond and Gordon 1997; National Institute on Alcohol Abuse and Alcoholism [NIAAA] 1997*a,b*; Peoples et al. 1996; West et al. 1994). In the developing brain, for example, alcohol has been shown to interfere with nerve cell development and function in a variety of ways (see the box, below). Thus, the brains of individuals with FAS show that certain regions have not developed normally, certain cells are not in their proper locations, and tissue has died off in some regions (for information about the functional consequences of these abnormalities, see the previous section, "Prenatal Alcohol Exposure: Effects on Brain Structure and Function"). These known actions of alcohol on the developing brain have provided scientists with numerous paths for identifying the biochemical mechanisms behind the actions, as described later in this section.

At a different site in the developing embryo—the cell layer that develops into the bones and cartilage of the head and face—alcohol exposure at critical stages of development induces the premature death of cells. This cell death, most

Actions of Alcohol on the Developing Nervous System

Alcohol interacts with the developing and adult central nervous system through multiple actions at different cellular sites. The list below outlines the major known actions of alcohol that provide candidate mechanisms of damage to the developing brain. Alcohol can:

- Interfere with the normal proliferation of nerve cells (Cook et al. 1990; Miller 1988, 1989, 1995, 1996; Pantazis et al. 1993).
- Increase the formation of free radicals—cell-damaging molecular fragments (Cedarbaum 1989; Chen and Sulik 1996; Davis et al. 1990; Henderson et al. 1995; Montoliu et al. 1995; Nordmann et al. 1992).
- Alter the cell's ability to produce or respond to factors that regulate cell growth, division, and survival (Bhave and Hoffman 1997; Crews et al. 1996; Cui et al. 1997; Deltour et al. 1996; Dohrman et al. 1997; Heaton et al. 1995*b*; NIAAA 1997*b*; Singh et al. 1996*b*; Valles et al. 1994).
- Impair the development and function of astrocytes—cells that guide the migration of nerve cells to their proper places (Guizetti et al. 1997; Miller 1993; Miller and Robertson 1993; Phillips and Krueger 1990; Valles et al. 1994, 1996; Zoeller et al. 1994).
- Interfere with the normal adhesion of cells to one another (Charness et al. 1994; Ramanathan et al. 1996).
- Alter the formation of axons—nerve cell extensions that conduct impulses away from the cell body (Dow and Riopelle 1985; Messing et al. 1991; Roivanen et al. 1995; Rosenberg and Noble 1994; Saunders et al. 1995; Zou et al. 1993).
- Alter the integrity and function of cell membranes (Chen et al. 1996; Devi et al. 1993).
- Alter the pathways of biochemical or electrical signals within cells (Davis-Cox et al. 1996; De et al. 1994; Diamond and Gordon 1997; Dohrman et al. 1996; Roivanen et al. 1995; Yang et al. 1996).
- Alter the regulation of calcium levels in the cell (Dildy and Leslie 1989; Gruol and Curry 1995; Webb et al. 1996*a,b*).
- Alter the expression of certain genes—in which the gene's encoded information is converted into a product such as a protein (Fletcher and Shain 1993; Miles et al. 1991)—including genes that regulate cell development and survival (Hogan and Barnes 1992; Rifas et al. 1997).

likely the product of several interacting biochemical mechanisms also described later, is thought to be linked to the facial abnormalities found in FAS.

A number of risk factors influence the degree to which alcohol exposure causes the different forms of fetal damage expressed in FAS. Through basic studies in animals and cell cultures, for example, researchers have shown that susceptibility to specific FAS defects appears to be directly related to the timing of maternal drinking, that is, whether drinking occurs during critical periods of vulnerability for different organ systems, regions, or cell types (Coles 1994; Goodlett and Johnson 1999; Maier et al. 1996). Animal studies have also shown that the type and extent of fetal damage are related to the pattern of maternal drinking, with binge drinking being particularly damaging (Goodlett et al. 1997, 1998); the particular profiles of blood alcohol concentrations produced (West et al. 1990); the duration of exposure during development (Maier et al. 1996); and differing levels of susceptibility related to the genetic makeup of the mother and fetus (Thomas et al. 1998). Moreover, the effects of alcohol may be enhanced by other conditions that adversely affect the fetus, such as the use of tobacco and other drugs by the mother (Abel and Hannigan 1995; Maier et al. 1996; Phillips et al. 1989) and abnormalities in the mother's physiology, including those caused by malnutrition (Polache et al. 1996).

In addition to directly affecting fetal tissues, alcohol can act indirectly through its effects on placental function and maternal-fetal blood flow (Altura et al. 1982; Falconer 1990; Karl and Fisher 1994; Phillips et al. 1989; Randall and Saulnier 1995; Randall et al. 1989; Savoy-Moore et al. 1989; Schenker et al. 1989, 1990; Siler-Khodr et al. 1996; Taylor et al. 1994). Moreover, although alcohol itself is generally considered to be the primary cause of FAS (Michaelis 1990; Michaelis and Michaelis 1994), a contributing factor may be the action of acetaldehyde, a by-product of the metabolism of alcohol (Hamby-Mason et al. 1997; Webster et al. 1983; Zimmerman et al. 1995).

To unravel the complex underlying mechanisms of FAS, scientists have needed to isolate specific aspects of FAS and investigate them in well-controlled studies. Progress in this field would not have been possible without research techniques involving animal models and tools of cellular and molecular biology (see the box "In Vivo and In Vitro Model Systems"). Results of these investigations have led researchers to propose a number of probable or "candidate" FAS mechanisms, which are described below.

Candidate Mechanisms for Central Nervous System Damage

Because the most disabling and permanent effects of FAS arise from alcohol's effects on the developing central nervous system (CNS)—the brain and spinal cord—a significant proportion of FAS studies have pursued mechanisms of CNS damage (see the chapter on neuroscience and neurobehavior for descriptions of the structural and functional components of the CNS). As noted, CNS damage can occur when alcohol interferes with the normal development and migration of nerve cells (neurons), disrupts cell functions, and causes cell death, either indirectly or by direct action on critical cellular components. Although some of the mechanisms underlying CNS damage are specific to nervous system tissue, others also affect development of the craniofacial region or other body areas. Regardless of the site of action, the timing, amount, and duration of alcohol exposure play a crucial role in determining the type and extent of damage.

Timing of Exposure

In the developing brain, alcohol exposure during various stages of development can harm different populations of neurons through different processes. Animal research has shown, for example, that if alcohol exposure occurs during the cell proliferation stage in early development, when brain cells undergo rapid division and growth, it can cause fewer cells to be generated (Miller 1995). If alcohol exposure occurs later, when the cells are differentiating and becoming specialized, some of the cells die after cell division (Miller 1995).

In Vivo and In Vitro Model Systems

Studies of the effects of alcohol on the developing embryo and fetus depend upon a variety of model systems using either living animals (in vivo studies) or cell cultures and embryo cultures (in vitro studies). With these systems, investigators are able to control and manipulate doses, timing and pattern of exposure, blood levels of alcohol, and routes of administration, as well as nutrition and the environment.

Research using animal models has shown that each of the major characteristics of human Fetal Alcohol Syndrome (FAS), including craniofacial abnormalities, growth deficiency, and abnormalities of the central nervous system, occurs in one or more of these animals, including mice, rats, chicks, and primates. Because different species, and even strains within species, show different degrees of vulnerability to alcohol, experimental results must be interpreted with a measure of caution (Becker et al. 1994, 1996; Melcer et al. 1995; Thomas et al. 1998). However, the most common animal models for FAS research—mice and rats—are very similar genetically to humans, and their biochemical processes are virtually the same.

Selective breeding techniques have produced strains of mice and rats in which individuals are virtually identical genetically. Thus, variations in responses between individuals of the same strain can be attributed to environmental causes, while variations between animals of different strains can be attributed to genetic causes. Selective breeding has also been used to produce paired strains in which animals that are otherwise identical will vary significantly in one particular trait. For example, several pairs of rat lines have been produced in which animals of one strain will voluntarily consume high quantities of alcohol, while animals in the otherwise identical paired strain will drink very little. (The section "Animal Genetic Studies on Alcoholism" in the chapter on genetic and psychosocial influences describes the development of these and other strains.)

Two recently developed animal models are "knockout" strains of mice and rats, where one specific gene has been inactivated, or knocked out, and transgenic strains, where a foreign gene is integrated into the animal's DNA. Because of the high degree of similarity between locations of specific genes on mouse and human chromosomes, a trait that has been genetically mapped in the mouse can be located fairly accurately in human chromosomes.

Cell cultures allow detailed manipulation and analysis of cellular and molecular processes in closely defined cell populations, such as cells from one specific area of the brain. Whole embryos of mice and rats can be grown in culture, and chick embryos can be studied through holes cut into the egg. These models allow researchers to analyze the molecular mechanisms involved in alcohol's effects on living cells. Although in vitro studies are providing important information on molecular mechanisms of action, caution must be used in extrapolating these findings to actual pathways in the whole organism.

Some types of neurons are extremely vulnerable during the early stages of differentiation and when synapses are being formed (Bonthius and West 1991; Goodlett and Johnson 1999; Goodlett et al. 1998; Marcussen et al. 1994). In other instances, neurons die when alcohol exposure either prevents them from migrating properly (Liesi 1997) or induces a delayed cell death that occurs after migration, even though exposure occurred before migration started (Cartwright et al. 1998).

It is likely that, at least in some cases, distinctly different mechanisms will be found to be responsible for different effects of alcohol at different stages of CNS development. In addition, it is possible that different forms of cell death (described next in this section) may be induced at different times of exposure or at different alcohol concentrations. Moreover, multiple mechanisms may operate simultaneously to produce abnormal cell development or cell death.

Cell Death Modes

Cell death is the endpoint of many of the FAS mechanisms described in this section. Although any number of events may lead to cell death, it ultimately occurs by one of two recognized pathways: necrosis, a reaction to injury or disrupted cell metabolism, or apoptosis, a "programmed" self-destruction that is necessary for normal development but can be triggered to

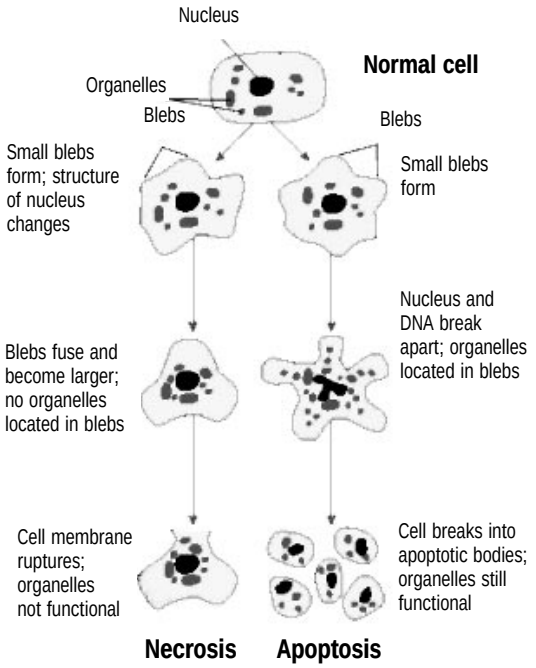
Cell Death Modes: Necrosis and Apoptosis

There are two modes of cell death: necrosis, which is a response to injury, and apoptosis, a form of "programmed" self-destruction where cells are induced to destroy themselves, apparently in response to instructions from their own genes. In the adult organism, apoptosis normally serves the purpose of maintaining a balance between the proliferation of new cells and the death of senescent or damaged cells. However, in the embryo this mode of cell death is necessary for proper development and shaping of tissues and organs, such as the removal of webbing between fingers and toes. Most cell death during normal development occurs by apoptosis, and apoptotic cell death is particularly important for normal development of the central nervous system. Whether prenatal alcohol exposure results in cell death by necrosis or by apoptosis depends on the severity, timing, and duration of the exposure.

Necrosis and apoptosis differ significantly in the biochemical and physical changes involved. During necrosis, the cell swells, metabolic functions cease both in the cell and in the intracellular organelles, and the cell membrane ruptures, releasing its contents into the surrounding tissue and causing inflammation. In contrast, apoptosis is an orderly process where the cell shrinks and the nucleus and the cell's DNA become fragmented, but the cell's metabolic processes continue. The cell develops small, bubble-like blebs on its surface and breaks up into small fragments called apoptotic bodies, each enclosed within a membrane and each containing still-functioning organelles. The process does not cause inflammation, and the apoptotic bodies are eventually absorbed by neighboring cells.

The signaling pathways that lead to apoptosis involve *bcl-2* genes, a family of genes that can either promote or inhibit apoptosis. The balance in expression of these genes regulates the "decision" between survival and cell death. The pathways to apoptosis involve the activation of death-promoting substances called caspases, which are enzymes that act as "executioners" by literally cutting apart the cell's proteins. It is likely that the apoptosis-inhibiting *bcl-2* genes act by inhibiting caspase activation. However, once the apoptosis-promoting cascade of reactions has begun, the inhibitory *bcl-2* genes usually cannot prevent cell death.

Figure 2: Modes of cell death



In necrosis (left), the cell forms bubble-like projections, or blebs, and the cell and its organelles become nonfunctional. The blebs fuse and become larger, but do not contain organelles. The cell membrane ruptures, releasing the cell's contents into the surrounding tissue, causing inflammation. In apoptosis (right), the cell similarly forms blebs, but the blebs contain still-functioning organelles. The nucleus becomes fragmented, and the cell breaks up to form apoptotic bodies, which remain metabolically functional. The apoptotic bodies are eventually engulfed by neighboring cells.

Source: Nanji and Hiller-Sturmhöfel 1997.

The mitochondria—intracellular organelles that play an important role in energy metabolism—may also play a role in the initiation of apoptosis. In response to certain signals that affect the mitochondrial membrane, the mitochondria may release cytochrome c and other substances into the cytoplasm. The cytochrome c, in turn, can activate genes that initiate the caspase cascade.

an excessive degree by toxins such as alcohol (Bredensen 1995, 1996*a,b*; Cartwright, et al. 1998; Ewald and Shao 1993; Wyllie et al. 1984) (see the box, above, and figure 2). While cell death by apoptosis is critical to healthy CNS development (Oppenheim 1991), this type of cell death also is involved in a broad range of human CNS disorders, including amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease) and Alzheimer's disease (Beal 1997).

Both modes of cell death, which can occur in response to the same toxin, appear to involve dysfunction of the cell's mitochondria (Keller et al. 1998; Kroemer et al. 1997; Schinder et al. 1996). The proportion of cells that follow each mode of death depends upon the intensity or duration of the toxic insult and upon the extent of mitochondrial damage (Ankarcrona et al. 1995; Bonfoco et al. 1995; Choi 1995; Keller et al. 1998; Kroemer et al. 1997; Pang and Geddes 1997). In addition, changes in the expression of certain genes (*bcl-2* genes) can determine whether or not cells die by apoptosis (Davies 1995; Li et al. 1997; Merry and Korsmeyer 1997; Reed 1997; Vaux and Strasses 1996; Yang et al. 1997). These and other recent advances in knowledge about cell death modes provide the basis for FAS studies on the role of alcohol in inducing cell death in developing tissues.

Free-Radical Damage

Free radicals are highly reactive molecular fragments that may be formed as a by-product of alcohol metabolism. It is very likely that formation of these fragments plays an important role in producing cell damage in FAS, both in the CNS and in the craniofacial region (Beal 1997; Cedarbaum 1989; Chen and Sulik 1996; Chen et al. 1997; Davis et al. 1990; Dykens 1994; Henderson et al. 1995; Montoliu et al. 1995; Nordmann et al. 1992). Free radicals can disrupt a cell's outer membrane or the membranes surrounding its organelles, such as mitochondria. In so doing, they upset the delicate balance of water, calcium, proteins, and other components within cells. Numerous studies have indicated that alcohol may damage or kill fetal cells by causing the breakdown of mitochondria (Devi et al. 1993, 1994), a process that can be initiated by excessive amounts of free radicals (Chen and Sulik 1996; Guerri et al. 1994; Henderson et al. 1995; Montoliu et al. 1994, 1995).

Antioxidants—such as vitamin C, vitamin E, and glutathione—are molecules that neutralize free radicals. Research has demonstrated that the addition of antioxidants to cell cultures can prevent cell death, suggesting the potential for therapies with antioxidant treatment (Chen and Sulik 1996; Chen et al. 1997; Davis et al. 1990; Reyes and Ott 1996; Reyes et al. 1993).

Interference With Growth Factor Functions

A number of chemicals, called growth factors, control cell proliferation and promote cell survival in the developing fetus (Henderson 1996). Current research indicates that alcohol exposure may disrupt the developing CNS by interfering with the production or function of some of these growth factors (Luo and Miller 1996, 1997; Resnicoff et al. 1993*a*,*b*, 1996). Described here are studies focusing on insulin-like growth factors, nerve growth factor, basic fibroblast growth factor, and a neurotrophic growth factor.

For cells to enter the stage of cell division in which the chromosomes are duplicated, the action of insulin-like growth factors (IGF-I and IGF-II) on the cells' IGF receptors is generally required (Rubin and Baserga 1995; Singh et al. 1996*b*). However, research has indicated that in the presence of alcohol, IGF-I binds with its receptors on the surface of neurons but is no longer able to stimulate cell proliferation (Resnicoff et al. 1993*a*,*b*, 1996). Studies on strains of mice that have been genetically engineered to inactivate the IGF-I receptors showed that intrauterine growth was severely stunted (Baker et al. 1993). IGF-I also supports the survival of nondividing cells and can prevent apoptosis in several types of cells, including specialized neurons in the cerebellum called granule cells (Galli et al. 1995). Recent studies have found that alcohol blocks this protective effect of IGF-I against apoptosis in granule cells (Zhang et al. 1998) and also in connective tissue cells called fibroblasts (Cui et al. 1997).

Evidence from studies using cultured cells of neural tumors (neuroblastoma) has supported these findings. Neuroblastoma cells that had been stimulated by growth factors showed that alcohol inhibited cell proliferation (Luo and Miller 1996, 1997). In contrast, neuroblastoma cell lines that did not normally respond to growth factors remained unaffected by alcohol (Resnicoff et al. 1996).

Two other growth factors—nerve growth factor and basic fibroblast growth factor—have also been shown to protect cultures of several types of brain cells from alcohol-induced death (Heaton et al. 1993, 1994, 1995*a*,*b*; Luo et al. 1997). Recent work has also shown that a growth factor called glial-derived neurotrophic factor protects against alcohol-induced cell death in cultures of specialized neurons of the cerebellum, called Purkinje cells (McAlhany et al. 1997). This protective effect appears to be an important clue into alcohol-induced loss of Purkinje cells, an effect that has been studied extensively in animals and for which issues of alcohol concentrations and stages of vulnerability are now relatively well understood (Goodlett and Johnson 1999).

Adverse Effects on Astrocyte Formation

Astrocytes are star-shaped cells of the nervous system that, unlike neurons, do not actively transmit information to other cells by communication across a synapse. Nevertheless, astrocytes interact intimately with neurons and other astrocytes and play critical roles in the developing CNS (Kettenman and Ransom 1995; Rakic 1991). One possible mechanism for alcohol-induced abnormalities in the fetus involves errors in the process of astrocyte formation.

Early in the fetal development of the brain, elongated cells that are precursors to astrocytes, called radial glia, act as tracks to guide migrating neurons to their appropriate destinations in the brain. Just as the period of neuronal migration ends, the radial glia normally transform into astrocytes and cease to provide tracks. In a study of rats, prenatal alcohol exposure caused radial glia to change into astrocytes prematurely, before the stage of neuronal migration was complete (Miller and Robertson 1993). This may explain why, in rats exposed to alcohol prenatally, neurons that develop late in the migration period were not found in appropriate places in the brain (Miller 1993).

More recently, researchers have found that alcohol interferes with the normal growth and function of astrocytes. For example, alcohol has been found to inhibit the normal proliferation of astrocytes in vitro (Guizetti et al. 1997; Holownia et al. 1997; Luo and Miller 1996) and in rats exposed to alcohol prenatally (Miller and Potempa 1990). Other in vitro studies found abnormalities in various aspects of astrocyte development (Guerri et al. 1993; Kim and Druse 1996*a*; Lokhorst and Druse 1993; Saez et al. 1991; Valles et al. 1996).

Depending on the stage of development, alcohol exposure causes different problems in astrocyte formation. As noted, when the exposure occurs during gestation, studies in rats have found diminished or delayed astrocyte development. However, when exposure occurs later, as modeled in a study of binge exposure in which alcohol was administered directly into the stomach of newborn rats (a developmental stage equivalent to the third trimester in humans), researchers found that the astrocytes became abnormally large and numerous and that protein levels increased (Fletcher and Shain 1993; Goodlett et al. 1993, 1997). These changes were dramatic but transient. In contrast, this reaction did not occur when newborn rats inhaled alcohol (Ryabinin et al. 1995). The reasons for these different reactions are not known, but the findings imply that alcohol exposure even late in human pregnancy may affect fetal astrocytes.

Abnormal Development of Neurotransmitter Systems

Neurons communicate via chemicals called neurotransmitters, which are released from an extension of the nerve cell body called the axon terminal. The neurotransmitter then travels across a narrow synaptic gap and binds to specific receptors on the target neuron. Research shows that alcohol has significant effects on two neurotransmitter systems that play important roles in fetal brain development: the serotonin system and the glutamate system.

Serotonin. An important step in the development of the cerebral cortex (the thin layer of tissue covering the cerebrum) appears to be the early embryonic growth of serotonin-releasing (serotonergic) neurons into the region that eventually develops into the cortex (Whitaker-Azmitia et al. 1996).

In studies of rats, very early prenatal exposure to alcohol significantly delayed the development of the serotonergic system, reducing serotonin levels and altering the binding of serotonin to receptors in many target sites during periods that are likely to be critical for normal brain development (Druse et al. 1991; Druse Manteuffel 1996). This effect may involve alcohol's interference with a process in which the developing nerve cells release serotonin, which stimulates specific receptors (called 5-HT_{1A} receptors) on neighboring astrocytes. These astrocytes, in turn, release growth factors that promote the growth, development, and survival of the nerve cells (Azmitia et al. 1990; Kim and Druse 1996*a*; Whitaker-Azmitia et al. 1990, 1996). Remarkably, one study found that treatment of pregnant rats with a medication that stimulates the same serotonin receptors—the antidepressant buspirone—protected against the alcohol-induced deficits in serotonin development (Kim and Druse 1996*b*).

The findings to date in this area suggest two important directions for future research: (1) characterizing the specific effects of alcohol-induced deficits in the serotonin system on the development of the cortex and other brain regions, and (2) identifying the serotonin-related mechanisms of nerve cell growth that involve the stimulation of astrocytes to release growth factors and the effects of alcohol on these mechanisms.

Glutamate. The neurotransmitter glutamate, considered the most important of the excitatory neurotransmitters (which increase neuronal activity), plays a major role in controlling brain function. During development, the activation of one type of receptor for glutamate—called the NMDA (*N*-methyl-D-aspartate) receptor—appears to be critical for establishing and stabilizing newly formed synapses, especially in the developing visual system and other CNS systems (Bear et al. 1990; Constantine-Paton 1994; Kirkwood and Bear 1994). Because alcohol is known to interfere with the function of NMDA receptors (Crews et al. 1996; Hoffman et al. 1989; Lovinger et al. 1990), exposure to alcohol during critical periods of synapse generation has been suggested as a likely mechanism for long-term effects on the organization of the CNS. (Glutamate and the NMDA receptor are extensively discussed in the chapter on neuroscience and neurobehavior.)

In contrast to the increase in number and sensitivity of NMDA receptors that occurs with chronic alcohol exposure in adult animals and in cell cultures (called up-regulation) (Follesa and Ticku 1996; Snell et al. 1996; Trevisan et al. 1994), prenatal alcohol exposure even in moderate concentrations results in a decrease in the number and function of NMDA receptors (called down-regulation) throughout development (Abdollah and Brien 1995; Diaz-Granados et al. 1997; Hughes et al. 1998; Lee et al. 1994; Morrisett et al. 1989; Savage et al. 1991; Spuhler-Phillips et al. 1997). Several studies in rats have shown that NMDA receptor down-regulation is most likely to occur when alcohol exposure occurs shortly after birth, a stage that correlates with third-trimester exposure in humans, and to last well past the period of alcohol exposure (Diaz-Granados et al. 1997; Gruol et al. 1998). Alcohol's effects on NMDA receptors during critical periods of brain development may play a major role in the mental and behavioral deficiencies found in FAS.

An important current avenue of study is the possible role of damage to the developing CNS from increased NMDA receptor activity that occurs during acute withdrawal periods associated with binge drinking (Thomas et al. 1998). One study in rats, using a model of binge exposure during CNS development, found that a medication that blocked NMDA receptor function (dizocilpine) lessened some of the long-term behavioral consequences of the alcohol exposure, such as hyperactivity (Thomas et al. 1997). Further studies are needed to identify the specific contribution of acute withdrawal effects to alcohol-induced brain damage.

Altered Glucose Transport and Uptake

Most cells of mammals contain specialized proteins that transport glucose from the blood

into the cells. Cells need glucose not only for energy metabolism, but also for metabolizing free radicals (Baquer et al. 1988) and for synthesizing vital chemicals, including neurotransmitters and nucleic acids. A number of studies have demonstrated that alcohol can impair glucose transport and uptake during development. For example, cell culture studies show that alcohol exposure can reduce glucose transporter protein levels and glucose uptake by certain brain neurons from fetal rats (Hu et al. 1995) and astrocytes from newborn rats (Singh et al. 1996*a*). These reductions also occurred in cells from the cerebral cortex during prolonged alcohol exposure in fetal rats (Singh et al. 1992). Studies on cultured rat embryos suggested that alcohol inhibited glucose transport in these embryos as well (Snyder et al. 1992). Other researchers, using chick embryos, found that alcohol reduced glucose uptake but increased insulin-stimulated uptake (Pennington et al. 1995) and altered some transporter proteins (Eckstein et al. 1997). Further research on the mechanisms of glucose transport and uptake could contribute significantly to knowledge about alcohol's effects on developing cells.

Abnormal Cell Adhesion Molecules

Cell adhesion molecules influence the ability of CNS cells to migrate properly, to develop branching extensions such as axons and dendrites, and to survive. Defects in one particular cell adhesion molecule, called L1, can lead to abnormalities in brain development and mental deficiencies (Wong et al. 1995) that are similar to those seen in children with FAS (Mattson and Riley 1996).

Some cell culture studies have shown that low levels of alcohol interfere with the ability of L1 to regulate the clustering or clumping together of cells that is needed for brain structures to develop (Charness et al. 1994; Ramanathan et al. 1996). The disruption of cell adhesion seems to depend on the type of cell culture used, however, as another study using a different type of cell culture found that alcohol did not interfere with L1-regulated cell clumping (Vallejo et al. 1997). Although the reasons for this discrepancy are not understood, additional research on the L1 defect mechanism may provide more insights into errors in cell migration, cell contact, and other aspects of FAS defects.

Altered Regulation of Gene Expression

The process of converting a gene's encoded information into a gene product (such as a protein) is called gene expression. In alcohol research, scientists are particularly interested in the expression of homeobox genes, which regulate the activation and timing of steps in the formation of specialized tissues and organs in the body (Hogan and Barnes 1992; Jonk et al. 1994; Marshall et al. 1996). Although it is known that alcohol can affect the expression of some genes, it is not yet certain whether these include homeobox genes.

In one study, a heavy dose of alcohol in pregnant mice at 7 days of gestation nearly eliminated the expression of a certain homeobox gene (called *msx2*) in the fetuses 3 days later and produced severely abnormal fetal growth (Rifas et al. 1997). However, it was not clear whether the lack of gene expression caused abnormal fetal growth or whether the abnormal growth prevented expression of the homeobox gene. Additional research will help elucidate this process.

The lack of information on how alcohol affects the regulation of genes that control the formation of the CNS and other body parts creates a major gap in our understanding of the mechanisms underlying FAS. (One exception is research on a mechanism involving retinoic acid production and craniofacial defects described next in this section.) In addition to homeobox genes, more knowledge is needed about alcohol's effects on genes that control cell survival and cell death, such as the *bcl-2* genes. Cell death can be blocked by *bcl-2* genes that inhibit apoptosis, presumably through inhibition of enzymes called caspases, the "executioners" that cut apart the cell's proteins (Cohen 1997; Du et al. 1997; Hara et al. 1997; Jung et al. 1996; Kane et al. 1993; Kermer et al. 1998; Nicholson et al. 1995; Parsadanian et al. 1998). Studies on alcohol-induced changes in gene expression during critical periods of development constitute one of the most promising areas for new FAS research.

Candidate Mechanisms for Craniofacial Defects

Animal studies have linked the characteristic facial abnormalities in FAS to cell death by apoptosis of certain embryonic cells, called neural crest cells, during a very defined and narrow period of vulnerability (the embryonic stages of gastrulation or neurulation) (Cartwright et al. 1998; Sulik et al. 1981). One mechanism by which this occurs is thought to be the formation of free radicals (Kotch et al. 1995). In studies of mouse neural crest cells, alcohol was associated with cell death due to the formation of free radicals, a process that could be prevented with antioxidants (Chen and Sulik 1996; Chen et al. 1997; Davis et al. 1990).

Two other possible mechanisms, described in more detail below, are a deficiency in retinoic acid and altered expression of homeobox genes. All three of these mechanisms are likely interrelated, since retinoic acid is a key regulator of gene expression, and both free-radical toxicity and altered gene expression can produce apoptosis. The effects of these mechanisms, as with those that damage the CNS, depend in part on the timing of alcohol exposure.

Timing of Exposure

In mouse and chicken embryos, exposure to alcohol during certain periods of development can give rise to the craniofacial abnormalities associated with FAS (Cartwright and Smith 1995*a,b*; Cartwright et al. 1998; Kotch and Sulik 1992*a,b*; Sulik and Johnston 1983; Sulik et al. 1981, 1988; Webster et al. 1983). In mice, a narrow period of vulnerability to craniofacial abnormalities was observed about 7 days after fertilization (Duester et al. 1996). Extensive research with chicken embryos also revealed that exposure to alcohol during narrow windows of vulnerability caused the death of neural crest cells by apoptosis (Cartwright and Smith 1995*a,b*; Cartwright et al. 1998). These researchers demonstrated that, to induce apoptosis, alcohol exposure must occur before the neural crest cells begin to migrate and that cells do not actually die until after migration.

Retinoic Acid Deficiency and Altered Gene Expression

Extensive evidence indicates that retinoic acid, a derivative of retinol (vitamin A), is essential for controlling the normal pattern of development of tissues and organs in vertebrate animals (Boncinelli et al. 1991; Durston et al. 1989; Hofmann and Eichele 1994; Hogan and Barnes 1992; Jonk et al. 1994; Mangelsdorf et al. 1994). Research shows that retinoic acid is necessary for the development of neural crest cells into craniofacial features (Morriss-Kay 1993; Morriss-Kay and Sokolova 1996) and strongly suggests that it acts in this capacity by binding with receptors that regulate the expression of homeobox genes (Hogan and Barnes 1992; Jonk et al. 1994; Marshall et al. 1996).

Ultimately, deficiencies or abnormalities in retinoic acid or its receptors cause neural crest cells to die by apoptosis, leading to craniofacial defects (Dickman et al. 1997; Grummer and Zachman 1995; Grummer et al. 1993; Henion and Weston 1994; Hofmann and Eichele 1994; Mangelsdorf et al. 1994; Morriss-Kay 1993; Morriss-Kay and Sokolova 1996).

In 1991, it was proposed that the craniofacial features of FAS are caused by low concentrations of retinoic acid in the embryo (Duester 1991; Pullarkat 1991). Research since then has shown that alcohol exposure at specific periods of embryonic development can reduce the production of retinoic acid (Deltour et al. 1996). Some studies also suggest that decreased levels of retinoic acid may contribute to alcohol-related heart defects (De Jonge and Zachman 1995; Twal and Zile 1997).

For cells to convert retinol to retinoic acid, the action of certain forms of the alcohol-metabolizing enzyme alcohol dehydrogenase (ADH) is required (Ang et al. 1996*a,b*; Duester et al. 1996; Kim et al. 1992; Zgombic-Knight et al. 1995). In a study of mice, one form of ADH (Class IV) first appeared 7 days after fertilization (Duester et al. 1996), which is also when retinoic acid is first produced (Ang et al. 1996*a,b*). Another study showed that the addition of a high concentration of alcohol

to cultures of mouse embryos during this same time frame—7 to 8 days after fertilization—caused a decrease in the amount of retinoic acid in neural crest cells (Deltour et al. 1996). These results suggest that, in neural crest cells, alcohol successfully competes with retinol to bind with Class IV ADH. In this way, alcohol limits the formation of retinoic acid during a critical period of embryonic development.

Several studies have found that certain retinoic acid receptors control the specific homeobox genes that regulate the timing and coordination of craniofacial development (Hogan and Barnes 1992; Jonk et al. 1994; Marshall et al. 1996; Rifas et al. 1997). Although alcohol has been shown to reduce retinoic acid levels, a recent study using chick embryos found that alcohol had no effect on the expression of the homeobox gene *msx2*, which is known to be involved in normal development of neural crest cells, nor did it affect a related growth factor (BMP4) (Cartwright et al. 1998). The BMP4-msx2 pathway is a signaling pathway that induces apoptotic cell death in neural crest cells (Davidson 1995; Graham et al. 1994). This finding contrasts with another recent study in which expression of the same homeobox gene in mouse embryos was dramatically altered by a binge pattern of alcohol exposure, leading to severe growth deficiencies (Rifas et al. 1997). Although species differences may explain the discrepancies, it is also possible that the lack of homeobox gene expression in the mouse cells was a result of massive apoptotic cell death following administration of alcohol. As mentioned earlier, future research on FAS mechanisms will need to fill the gaps in our understanding of alcohol-induced changes in gene expression.

In Closing

Advancements in our understanding of mechanisms of FAS damage will guide the development of new ways to protect against or limit alcohol-induced damage to the fetus. Opportunities exist, for example, to identify windows of time in which treatments may block specific types of damage or rescue otherwise vulnerable cell populations. Identification of specific mechanisms and biochemical markers of damage should accelerate early detection or allow better prediction of specific types of damage in at-risk pregnancies. Such advances could help to identify cases at greatest risk for developmental disorders and to improve outcomes through targeted interventions.

Clarifying the mechanisms of brain damage in FAS should yield insights into the long-term adaptations of CNS cells and the potential for neuronal plasticity, in which neurons surrounding an injury change their synaptic connections to compensate for cell death or injury. Advances in knowledge about these long-term CNS adaptations could provide a basis for therapeutic approaches to the problems of long-lasting deficits in behavior and learning that are typical of FAS. From a public health perspective, knowledge of specific mechanisms of damage should be a powerful tool for effective public education and counseling of alcohol-dependent women in their childbearing years and could help guide clinical decisions about the most effective allocation of medical and psychological support services.

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